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Viruses in Drinking Water

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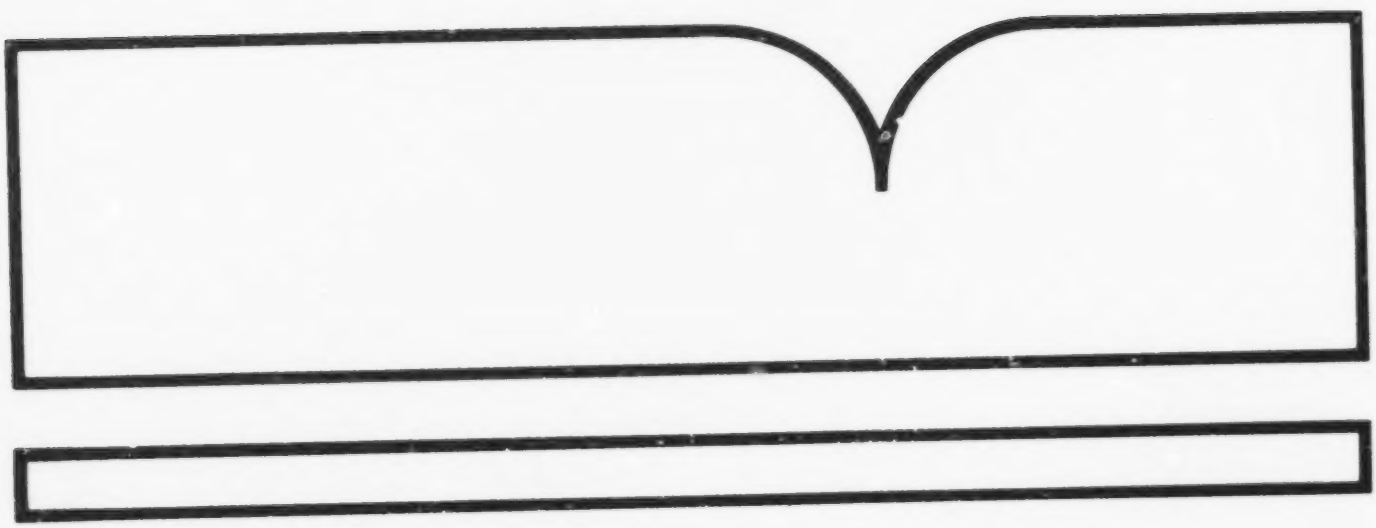

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16. ABSTRACT

Rising populations around the globe have resulted in an ever-increasing need for potable waters. Communities obtain their potable water from surface or underground sources. The contaminations of these source waters with viruses and other pathogens and parasites has been well documented. The source waters, particularly groundwater, are sometimes consumed without any treatment or after being only disinfected. They are also subjected to the full treatment provided by conventional water treatment plants. Do these plants provide a safe barrier against virus breakthrough? Are classical water quality parameters adequate for guaranteeing safe drinking water from virological standpoint? What are the priorities with respect to research on viruses in drinking water? To attempt to answer some of these questions, we will review the available literature dealing with virus monitoring of drinking water and then make comments on detection methods, indicators, effect of water treatment processes on virus removal and finally epidemiological health significance of viruses in drinking water.

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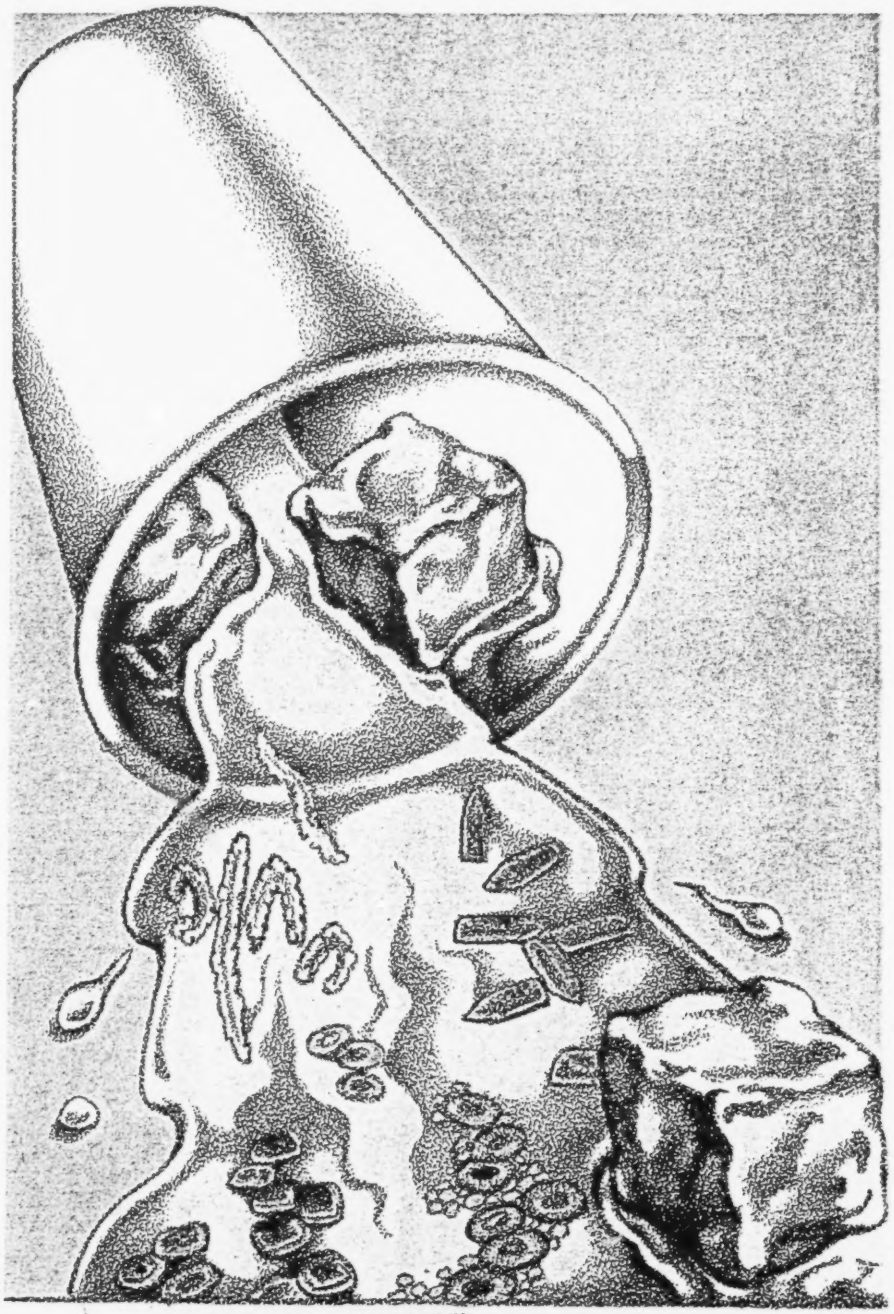
CRITICAL REVIEW

Viruses in drinking water

A global survey shows that although viruses are often present in treated drinking water, setting standards may be premature



Gabriel Bitton
Samuel R. Farrah
Clay L. Montague
University of Florida
Gainesville, Fla. 32611

Elmer W. Akin
Environmental Protection Agency
Cincinnati, Ohio 45268

The rising world population has resulted in an ever-increasing demand for potable water, which in most cases is obtained from surface or underground sources. The contamination of source waters with viruses and other pathogens and parasites has been well documented. The source waters, particularly groundwater, are sometimes consumed without any treatment or after disinfection only. In many cases, however, they are also subjected to the full treatment provided by conventional water treatment plants.

There are a number of questions to be asked about drinking water contaminated with viruses:

- Do treatment plants provide a safe barrier against virus breakthrough?
- Are classical water quality characteristics adequate to guarantee safe drinking water from a virological standpoint?
- What are the research priorities with respect to viruses in drinking water?

In an attempt to answer these questions, we will review the available literature on the monitoring of viruses in drinking water and then comment on detection methods, indicators, the effect of water treatment processes on virus removal, and the epidemiological significance of viruses in drinking water.

Available data

The findings of Coin and his collaborators in Paris, France, undoubtedly stimulated environmental virology in

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general and the search for viruses in drinking water in particular. They isolated polioviruses, coxsackieviruses, and echoviruses from 9% of the drinking-water samples they took in Paris (*1*). Subsequently, Coulon and Netter reported that they were unable to isolate any viruses from Paris drinking water that had been treated via coagulation, filtration, and ozonation (*2*). Coxsackie B5 and reoviruses were found in the drinking water of an unnamed French city that treated its river water by flocculation–sedimentation, filtration, and chlorination or ozonation (*3*).

Later, Foliguet et al. reported the presence of enteroviruses and a chlorine residual in 11% of their drinking-water samples (*4*). Agbalika et al. (*5*) and Hassen (*6*) monitored the water treatment plant in Nancy, France, where the sequence consists of preozonation, storage, flocculation, sand filtration, activated carbon treatment, and ozonation.

Although enteroviruses were detected in the source water from the Oise River, none were found in 800–1000-L samples of treated drinking water. Similarly, Vilagines (unpublished data) did not detect any virus following monthly sampling from 1981 to 1983 of drinking water from three Paris water treatment plants that showed a chlorine residual of 0.5–0.6 ppm. In two of the plants ozone was used at 0.4 ppm for 10 min contact time prior to chlorination.

More recently, Festy (unpublished data) released data on virus isolations from Paris drinking water for 1975–83. The source water was drawn from the Seine and Marne rivers or from underground sources. Six of 819 samples were positive for enteroviruses. It is worth pointing out that the drinking-water samples consisted of 6000 L of water concentrated on gauze pads.

U.S. experiences

Following some controversial reports on virus isolation from drinking water in the United States, EPA conducted a survey of viruses found in the drinking water of 54 communities across the country (*7, 8*). Most water treatment plants in the survey had full conventional treatment with flocculation–sedimentation, sand filtration, and disinfection. No virus was found in any of the samples despite the relatively large volumes of water sampled (200–1900 L/sample).

More recently, a water treatment plant in Lexington, Mo., was monitored for viruses over a 2-y period (*9*). Relatively low concentrations (1.5–10 PFU [plaque-forming units]/380 L) were found in the source water, but no viruses were detected in the 71 finished water samples (1900 L each) following treatment by flocculation–sedimentation, lime treatment, filtration, and disinfection. It can be argued that the inability to detect viruses in the finished water may be due partly to the limitations of the available procedures, because a relatively low virus load was detected in the highly polluted source water.

Stetler et al. found a water treatment plant in southern Michigan that had relatively high virus levels in the source water (3–293 PFU/380 L) and excessive levels of trihalomethanes (THMs) in the finished water (*10*). The treatment sequence consisted of prechlorination, flocculation–sedimentation, sand filtration, and storage. This sequence was modified to decrease THM formation in the finished water. Prechlorination was discontinued and chlorination was moved to the last step of water treatment. No viruses were recovered from the finished water.

This is not the case for marginally treated drinking water, as illustrated by the isolation of viruses from potable waters associated with outbreaks of gastroenteritis in the United States. Following one such episode reported among migrant workers in Florida, echovirus 22/23 was isolated from the drinking-water supply, which was chlorinated groundwater (*11*). Following another outbreak in Georgetown, Tex., Hejkal et al. isolated coxsackievirus B3 from the chlorinated groundwater (drawn from 57–60-m-deep wells in a limestone area) (*12*). As a result of a faulty chlorinator, coxsackievirus B3 was isolated from the drinking water on a California Indian reservation (*13*).

Foreign experiences

Because concentrations of viruses are relatively low in source waters to conventional treatment plants in the United States, researchers have explored the possibility of studying the fate of viruses in water treatment plants in locations where concentrations of the viruses may be higher. Deetz et al. examined drinking water from Guadalajara, Mexico; enteroviruses and rotaviruses were isolated in 20-L samples of drinking water (*14*). A more extensive investigation was undertaken on the same treatment plant (*15, 16*). Samples of finished water harbored enteroviruses and rotaviruses despite acceptable levels of residual chlorine. Enteroviruses and rotaviruses were also detected in finished water in Puerto Rico despite turbidity of <1 NTU (nephelometric turbidity unit) and a free-chlorine residual of 1.5 ppm. Reoviruses and hepatitis A virus (HAV) were not found in the finished water (Gerba et al., unpublished data).

In Canada, Sekla and co-workers released the first report on virus isolation from drinking water (*17*). The three strains of polioviruses (all vaccine strains) were detected in drinking water from Manitoba in the presence of a chlorine residual of 0.1–0.2 mg/L. Poliovirus 1 was also found in 1000-L samples from the Pont Viau water treatment plant located in the Montreal, Que., area (*18*).

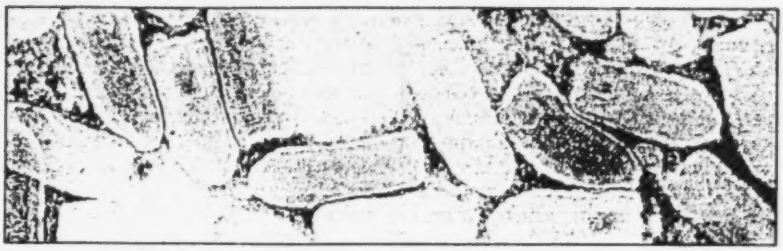
In this case, the viruses were detected as a result of operational problems at the water treatment plants, according to Payment (unpublished data). Virus monitoring in nine water treatment plants in three major Canadian urban areas (Montreal, Que., and Ottawa and Toronto, Ont.) did not show the presence of viruses in treated drinking water from any of the plants. However, it was argued that the BSC-1 host cells (monkey kidney cells) used in the virus assays during the collaborative study were not efficient in isolating viruses from drinking water (*19*).

Payment's group undertook another virus survey using BGM and Vero cells to isolate the viruses (*20*). Enteroviruses were detected in finished waters from five of seven plants. Viruses were found in finished water from plants where full treatment was used, including prechlorination, coagulation–sedimentation, filtration, ozonation, and postchlorination.

In South Africa, Nupen recovered viruses from five of one hundred 10-L samples of conventionally treated drinking water (*21, 22*). Extensive work has been carried out in that country on wastewater reclamation systems at the Windhoek and Stander plants. Grabow et al. reported the isolation of an unidentified enterovirus in one of 117 samples taken from the Windhoek plant (*23*). According to the authors, the virus was most likely a contaminant that had been introduced into the water during sampling.

In Israel, Guttman-Bass and Fattal (unpublished data) surveyed drinking-water supplies in 30 agricultural settlements for the presence of viruses. One hundred eleven samples (100–300 L/sample) were taken over an 18-month period. Three samples were found positive for enteroviruses but none of them contained residual chlorine.

In Great Britain, Slade reported that no virus was found in drinking water following treatment consisting of two to six weeks' storage, rapid and slow sand filtration, and final chlorination (0.5 ppm chlorine residual and 1-h contact time) (*24*). More recently, Tyler reported examinations of 553 samples of potable and distribution waters between 1979 and 1982 (*25*). She found that 16% of the samples contained po-



lioviruses, coxsackie B viruses, and echoviruses. Sixty per cent of the isolates were polioviruses. Viruses were found in drinking water with a chlorine residual exceeding 0.3 ppm. She reported, however, that on at least three occasions the chlorine residual dropped sharply prior to sampling. This drop may have been the result of an increase in turbidity following a heavy rainfall.

Viruses have been found in the distribution systems of Moscow and Kuybishev in the USSR (*26*). However, no viruses were found when a new modification to the chlorination practice resulted in the requirement of 0.3 ppm free residual chlorine after 30 min contact time. At a recent French–Soviet symposium, Kazantseva and Drozdov reported the presence of enteroviruses in 17% of drinking-water samples (*27*).

In Romania, Nestor and his collaborators isolated coxsackieviruses in two of 65 drinking-water samples after flocculation, sand filtration, and disinfection (*28, 29*). During a limited survey, Walter et al. found one positive sample (an unidentified enterovirus) out of four samples of chlorinated well water from East Germany (*30*).

In the Netherlands, source water for treatment plants is drawn from the Rhine and Meuse rivers. The treatment generally includes storage in reservoirs for at least a month, followed by dune filtration, coagulation–sedimentation, sand filtration, ozonation, and chlorination. Kool and Van Kranen surveyed six Dutch water treatment plants for a 2-y period (*31*). Although 10 of the 100 drinking-water samples (500 L/sample) were found positive for viruses, the authors stated that there was cross contamination with raw-water concentrates. Another survey of eight water treatment plants in the Netherlands did not reveal the presence of any viruses in the 500-L samples (*32*).

In Italy, viruses have been found in chlorinated groundwater used for drinking (*32*). However, no viruses were detected in water samples from a waterworks that treats stream water by prechlorination, coagulation–sedimentation, sand filtration, and postchlorination with chlorine dioxide (free chorine residual of 0.1 ppm; 30 min contact time).

In Spain, Pares et al. reported the isolation of reoviruses 2 and 3 from nonchlorinated potable water (*34*). On Norfolk Island in the South Pacific, where gastroenteritis is endemic (*35*), enteric viruses (polioviruses, adenoviruses, and rotaviruses) were found in concentrates from 5-L samples of bore water used for drinking (*36*).

Zhang et al. isolated coxsackieviruses and adenoviruses from 1-L samples of drinking water in Wuhan in the People's Republic of China (*37*). All the samples taken were positive for viruses. Unfortunately the authors did not provide any data on the method of water treatment.

Since the infamous outbreak of waterborne hepatitis in Delhi, India, Indian environmental virologists have become aware of the need to learn more about viruses in drinking water. Using a concentration method based on adsorption to iron oxide, Rao and collaborators found viruses in drinking water despite 0.2–0.8 ppm residual chlorine (*38*). Enteroviruses were isolated from seven of 50 samples.

In extreme situations, such as those found in Ghana, water from ponds, rivers, swamps, and wells is consumed without any treatment. Otatume and Addy reported the isolation of enteroviruses in 1- and 2-L samples from various locations (*39*).

Additional information on viruses isolated, host cells used, concentration methods, sample volumes, and simultaneous isolation of traditional microbial indicators is given elsewhere (*40*).

Detecting viruses

Because viruses can occur in small concentrations in conventionally treated drinking water there is a need to sample relatively large volumes—of 100 L or more—to detect their presence. The numerous concentration procedures for viruses in water have been described in detail (*41, 42*).

The most commonly used procedure is based on the adsorption of viruses to negatively charged filters (such as Balston, Cox, or Filterite filters) or to positively charged filters (such as Zeta+ and Virosorb 1-MDS filters). In early surveys viruses were eluted from the filter surface with glycine at pH 11.5. In more recent surveys viruses have been eluted with beef extract at pH 9.0–9.75 and further concentrated by the organic flocculation procedure of Katzenelson et al. (*43*).

French and Spanish investigators routinely use the glass powder method developed by Sarrette et al. (*44*). The mean volume processed is 200 L. Gauze pads were used in early studies (*45*). Festy (unpublished data) recently used gauze pads for concentrating 6000-L samples of tapwater from Paris. The pad extracts were further concentrated by membrane filtration. Other less frequently used methods are ultrafiltration (*36*), ultracentrifugation (*39*), adsorption to iron oxide (*38*), and adsorption to aluminum hydroxide flocs (*46*). These procedures have generally been used for relatively small volumes (1–25 L) of marginally treated water.

The evaluation of concentration methods for rotavirus recovery from drinking water was first attempted by Ramia and Sattar in seeded studies using simian rotavirus SA-11 (*47*). The virus was recovered from 100 L of drinking water by a talc–celite method followed by hydroextraction. Other investigators have used membrane filtration procedures to concentrate rotaviruses from seeded tapwater (*48, 49*). The recovery efficiency is generally similar to that found for enteroviruses.

During the 1980 hepatitis outbreak in Georgetown, Tex., hepatitis A antigen was detected via radioimmunoassay using a concentration procedure consisting of adsorption to Filterite followed by elution with beef extract and ultrafiltration (*12*). It appears that this virus can be concentrated from drinking water by methods developed for enteroviruses. Subsequent seeding studies using the HM175 strain support this conclusion (*50*). No concentration method has been developed for the detection of Norwalk virus in environmental samples.

Finally, a concentration procedure for monitoring viruses in conventional water treatment plants should be able to process relatively large volumes of water and should be gentle enough to avoid virus inactivation during processing. Selected microporous filters with adequate elution, for example using beef extract at pH 9, should be suitable for this task.

Indicators of virus presence

In certain instances, viruses may be present in finished drinking waters because they sometimes pass through water treatment plants. Virus detection in drinking water requires concentration from relatively large volumes of water and time-consuming and expensive assay procedures. This has led to a search for the best indicators for viral presence

in drinking water and other environmental samples (*51, 52*).

The indicators under consideration are the classical indicators of fecal pollution (total and fecal coliforms, fecal streptococci, and standard plate count at 22 °C and 37 °C), anaerobic bacterial indicators (*Clostridium perfringens* and *Bifidobacteria*), acid fast bacilli (*Mycobacterium fortuitum* and *Mycobacterium phlei*), and bacterial phages.

There have been numerous debates about the usefulness of these microorganisms for indicating the safety of drinking water from a virological standpoint. Some argue that viral infections are rarely caused by drinking water that does not meet coliform standards (*53*). Others contend that there are serious limitations to the use of coliform bacteria as a viral indicator (*54*). There are considerable variations in the ratio of bacterial indicators to virus numbers. These fluctuations may be due to the fact that viruses are excreted in variable numbers by infected individuals, or they may be the result of differences in the survival ability of viruses and bacteria and the possibility that some bacterial indicators multiply in environmental waters.

In many instances, the presence of surrogates indicates the presence of viruses. However, their absence does not ensure the absence of viruses (*54*). Despite these limitations some authors have found that in certain instances specific groups of indicators may be useful for determining the virological safety of water. For example, in wastewater reclamation plants in South Africa, Grabow et al. reported that the total plate count was the most sensitive indicator of the efficiency of wastewater reclamation plants, particularly during the final disinfection step (*55*). Coliphages occur in water and wastewater in higher numbers than animal viruses do. Their structural and morphological similarity to animal viruses and their higher resistance to some water and wastewater treatment processes have led some to suggest that they may be suitable as indicators of viral presence. Furthermore, coliphage assays are easy, rapid, and inexpensive (*51*).

A recent literature survey revealed that in most cases there appears to be no relationship between viruses and microbial indicators (*40*). Viruses were found in drinking water considered safe from a bacteriological standpoint. In the few studies where the data were statistically analyzed, no correlation was found between viruses and standard plate count, total coliforms, fecal coliforms, fecal streptococci, *Staphylococcus*, and *Pseudomonas* species (*20*).

In a study of water treatment plants in Guadalajara, Mexico, Gerba and his collaborators found that coliphages are significantly correlated with rotaviruses (*15*). Schwartbrod (unpublished data) detected fecal streptococci and coliphages in a 200-L sample that was shown to be positive for reovirus 2. Similarly, Keswick et al. concluded that fecal streptococci appear to be the best indicators of viral presence in drinking water (*16*).

There are many instances in which drinking-water samples were negative for viruses and microbial indicators (*6, 7, 10, 31, 32*). Some of the discrepancies may result from some investigators' using 50- to 100-mL volumes for bacterial and coliphage detection, whereas others have used 0.5- to 1-L volumes. It also seems unreasonable to compare bacterial and coliphage numbers found in 100-mL samples with virus numbers found in 500–1000-L samples of drinking water. A study group of the International Association of Water Pollution Research and Control has recommended an increase in the standard volume of water used for coliform analysis (*53*).

A similar recommendation can be made for coliphages because they may be concentrated by membrane filtration procedures. If these recommendations prove helpful in indicating the presence of virus in water, it will be desirable to develop simple, quick, and inexpensive procedures for concentrating coliphages and bacterial indicators in drinking water.

Drinking water that meets U.S. standards basically must have < 1 NTU turbidity and 0 coliforms/100 mL (*56*). Although not a standard, some authorities recommend that drinking water should be chlorinated at 0.5–1.0 mg/L for 30 min prior to distribution (*57, 58*). There are reports documenting virus isolation in drinking water that does not meet the chlorine residual recommendations (*3, 17,* and Guttman-Bass and Fattal, unpublished data). However, there are numerous reports that describe detection of viruses in drinking water that is acceptable for chlorine residual and turbidity (*14–16, 19, 25, 38, 58, 59,* and Gerba et al., unpublished data). Gerba et al. isolated viruses in 24% of drinking-water samples that met U.S. standards (*15*). In one of the plants monitored by Payment et al. in the Montreal area, viruses were found in finished drinking water when the mean values for turbidity and free chlorine residual were 0.4 NTU and 0.8 mg/L, respectively (*20*).

It may be that the sampling regimen is not refined enough to detect occasional changes in turbidity and chlorine residual. Suboptimal operating conditions in the water treatment plant (for example, poor floc formation) may contribute to the decreased chlorination efficiency resulting from temporary increases in turbidity.

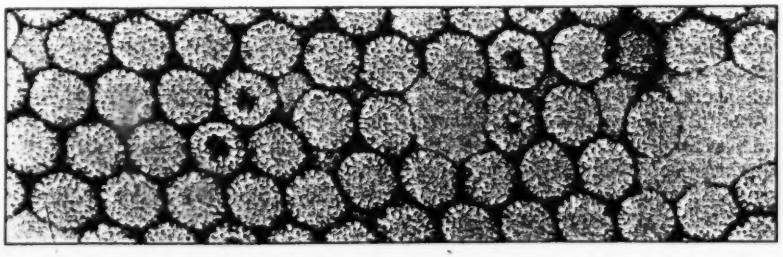
Heavy rains also may lead to increased turbidity with a concomitant decrease in chlorination efficiency. This point is well illustrated by the work of Tyler (*25*). She isolated viruses from water with a chlorine residual above 0.3 ppm. However, a retrospective examination of the chlorination process showed that the chlorine residual had dropped to trace levels a few hours prior to sampling.

Therefore, short-term changes in operating or climatic conditions may contribute to occasional virus breakthroughs even in well-operated water treatment plants. This point deserves consideration in the planning of future studies on the fate of viruses in water treatment systems.

Virus removal

There are two kinds of water treatment plants: conventional filter plants and softening plants. In filter plants the treatment steps generally involve prechlorination, coagulation–sedimentation, sand filtration, and disinfection with chlorine or ozone. The operation of softening plants includes the softening process (addition of lime and sodium carbonate), sedimentation, filtration, and disinfection.

Data on virus removal by water treatment processes under laboratory conditions have been extensively reviewed by Bitton (*52*). The research data can be summarized as follows: Coagulation with aluminum and iron salts, if properly conducted, can achieve better than 99% removal of viruses. The efficiency of the coagulation–sedimentation process may be improved by the addition of coagulant aids such as cationic polyelectrolytes. Slow sand filtration is more effective than rapid sand filtra-



tion. However, the efficiency of rapid sand filtration can be improved when filtration is preceded by chemical flocculation (*60, 61*). Slow sand filtration was found effective in virus removal in experiments carried out in East Germany (*62, 63*).

In water-softening plants, the lime-soda ash process achieves better than 99.9% virus removal. In this process viruses are significantly inactivated (if the pH is maintained above 11) and physically removed by adsorption to magnesium hydroxide flocs. Disinfection, the last line of defense against virus breakthrough, effectively destroys viruses under proper conditions. Its importance in virus inactivation has been discussed elsewhere (*24, 52*). If properly conducted, disinfection is the most effective of the water treatment processes discussed previously for virus control.

Laboratory studies have generated data on the extent of virus removal or inactivation by the various water treatment processes. However, it is known that laboratory-grown and indigenous viruses may behave differently in water and wastewater treatment plants. For example, due to aggregation and association with particulate matter, indigenous viruses may be less effectively destroyed during the disinfection step.

Recent field studies have been undertaken to assess the extent of removal of viruses and microbial indicators by conventional filtration plants in Canada, Mexico, and the United States (*10, 15, 16, 20, 64*). Payment and co-workers have examined several water treatment plants in the Montreal area for removal of viruses and traditional bacterial indicators (*20, 64*). They concluded that the prechlorination–coagulation–sedimentation sequence achieved satisfactory removal of bacteria and viruses. Prechlorination alone did not appear to improve water quality, and this practice should be reevaluated because it may result in the formation of THMs (*10*). The Canadian researchers also observed that viruses were less effectively removed than bacterial indicators were.

The Canadian results are supported by investigations conducted in Mexico (*15, 16*). Gerba et al. found 81% removal for viruses, compared with more than 98% for coliphages and bacterial indicators (*15*). The problem is further complicated by the differences in removal efficiency of various groups of enteric viruses. For example, a survey of a water treatment plant in Montreal resulted in the isolation of enteroviruses only, although the source water contained large amounts of reoviruses (*20*). Furthermore, Gerba et al. observed that rotaviruses are not removed to the same extent as enteroviruses by chemical flocculation and sand filtration (*15*). Chlorination was found to be the most effective means of inactivating rotaviruses.

No field data are available on the virus removal efficiency of water-softening plants, and pilot plants should perhaps be used to study their performance and their ability to remove the viruses from drinking water.

Epidemiological significance

Viruses occasionally are detected in drinking water from conventional water treatment plants that meet the standards currently used to judge treatment efficiency. Their breakthrough may be due to relatively drastic changes in source water quality or to equipment and process failure. The significance of the presence of low numbers of viruses in drinking water remains an important question.

There has been considerable debate over the need to establish viral standards (*65, 66*). Some believe that because the minimum infectious dose for viruses is low (~1 PFU) there is a low level of waterborne transmission of viral diseases. It is hypothesized that this low-level transmission remains undetected because of the insensitivity of conventional epidemiological procedures (*7, 24, 67, 68*).

Reasons for the insensitivity of epidemiological methods include poor reporting of the diseases, the small number of clinical cases among infected individuals, the difficulty in distinguishing among symptoms caused by more than 100 enteric viruses, long incubation periods for some diseases, and contact transmission of the diseases from one person to another, which may obscure the role of drinking water in disease transmission (*53, 68, 69*). A World Health Organization committee recommended that drinking water be free of viruses and that 100–1000-L samples be tested for the presence of viruses (*24*). To date, there is no U.S standard for viruses in drinking water. Some researchers, particularly in Europe, doubt the importance of the role of drinking water in the transmission of viral diseases.

Feachem et al. stated that "there is no evidence for the existence of low-level waterborne transmission and that, even if it did exist, it might make no significant contribution to the maintenance of endemicity of enteroviral infections" (*65*). Foliguet argued that no epidemic outbreak has been linked to conventional water treatment plants that meet bacterial standards (*67*). These arguments are supported by the

recent findings that at least for healthy adults the minimum infectious dose for viruses may actually be much higher than 1 PFU (*70, 71*). However, low concentrations of viruses in drinking water may cause infections in a community over an extended period.

Epidemiological evidence of waterborne transmission is limited to HAV, Norwalk agent, and other gastroenteritis viruses such as rotaviruses (*72*). Infectious hepatitis has been conclusively shown to be waterborne. However, waterborne outbreaks of hepatitis account for less than 1% of all cases in the United States (*73, 74*). No data are available on the efficiency of HAV removal or inactivation in conventional water treatment plants.

Furthermore, there are only limited data on the effects of chlorination on HAV. Early studies showed that at least 0.4 ppm free residual chlorine was needed to destroy HAV (*75*). A comparative chlorination study showed that HAV is more sensitive to chlorine than are mycobacteria and poliovirus 2, but more resistant than indicator bacteria, reovirus 2, and rotaviruses (*76*). A more recent study revealed that a higher level of chlorine residual is needed to destroy HAV (*77*).

Norwalk virus is a major cause of gastroenteritis outbreaks in the United States (*78*). Many of these outbreaks have been attributed to insufficiently treated drinking water from surface or underground sources (*72, 79, 80*). There is no known host cell for the successful cultivation of this virus in the laboratory. Until one can be found it will be difficult to assess the fate of Norwalk virus in water treatment plants.

Rotaviruses belong to the reoviridae family and are the causative agents of countless cases of infantile gastroenteritis around the globe, and they are the suspected cause of waterborne gastroenteritis outbreaks in numerous countries (*72*). Rotaviruses have been detected in conventionally treated drinking water (*14–16*) and in groundwater used for drinking (*36*). As a result of seeded studies much is known about the survival of simian rotaviruses in the environment and their fate following water and wastewater treatment processes, but almost nothing is known about human rotaviruses. Although the latter can be replicated on tissue culture in the laboratory (*81, 82*), they have not yet been successfully isolated directly from environmental samples.

To make a realistic assessment of the potential role of drinking water in the waterborne transmission of viral diseases, more knowledge is needed about the behavior of HAV and gastroenteritis viruses in water treatment plants. The technology to concentrate viruses from large volumes of water is basically similar to that used for enteroviruses. Efforts should be directed toward developing rapid and sensitive assays, particularly for the Norwalk virus. The EPA is funding in-house and extramural research studies directed at achieving this goal.

We conclude that it is probably too soon to establish standards for viruses in drinking water, and the cost involved in implementing such standards could override any potential benefits. Risk–benefit analyses should help determine the acceptable risk associated with virus presence in drinking water.

Recently, a cost–benefit analysis for drinking water was conducted by Clark et al. (*83*). Both the "net benefit" and the "cost per life saved" approaches were used in the analysis. As a result of the cost analysis the researchers concluded that drinking water treatment at its best—including activated carbon treatment to remove carcinogenic chlorinated organics—was relatively less expensive than other preventive public health measures. Baker et al. studied the economic impact of a waterborne gastroenteritis outbreak that infected 61% (8800 residents) of the population of Sewickley, Pa. (*84*). Direct and indirect cost calculations led researchers to stress the importance of prevention in protecting drinking-water supplies from contamination.

What is ahead?

A review of the literature on monitoring the viral content of drinking water in various countries reveals that the key question of the health significance of low concentrations has not been answered. There is little information about the relationship between virus isolation in drinking water and resulting waterborne cases of viral disease. Virus isolation has been documented in partially treated drinking water from marginally operated and even from apparently well-operated water treatment plants. The findings point to the possible need for revising the classical water quality indicators such as turbidity, free-chlorine residual, and coliform content.

There is still the challenge to find a practical and sensitive indicator for viruses. At the most, only weak correlations have been found between viruses and microbial indicators in the few studies in which the data have been statistically analyzed. Current water quality indicators should be compared with viruses under more realistic conditions (for example, by increasing the volume of samples processed for indicators). The findings also point out a need for more knowledge of the effect of variations in source water quality, equipment, and process performance on virus removal by water treatment plants. Plant operation may need modification to deal with these problems.

The epidemiological reality of waterborne disease occurrence compels us to obtain more information on the detection of HAV, Norwalk agent, and human rotavirus in various stages of conventional water treatment. To achieve this, researchers must develop or improve concentration procedures and assay systems for these viruses.

Risk–benefit analyses will likely be important in evaluating the economic reality of the problem of viruses in drinking water. Such studies may help to determine whether the developed nations have reached a point of diminishing returns in water quality improvement or whether public health would be significantly improved by greater efforts in this area. Certainly, for developing nations, where viruses are sometimes found in drinking water, the case is clear: There is a need to upgrade the quality of drinking water. However, setting standards at this point is not realistic and should be delayed until some of the questions raised in this discussion have been answered.

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References

(1) Coin, L. et al. In *Advances in Water Pollution Research;* Jaag, O.; Baars, J. K., Eds.; Pergamon Press: Oxford, U.K., 1965.
(2) Coulon, G.; Netter, R. *Bull. INSERM* **1967**, *22*, 941–56.
(3) Foliguet, J. M.; Schwartzbrod, L.; Gaudin, O. G. *Rev. Hyg. Med. Soc.* **1966**, *14*, 411–32.
(4) Foliguet, J. M. et al. *Public Health Aspects of Viruses in Water,* newsletter; Clarke, N.A., Ed.; Cincinnati, Ohio, 1981.
(5) Agbalika, F. et al. Presented at the International Association of Water Pollution Research Control Conference, Amsterdam, the Netherlands, 1984.
(6) Hassen, A. Ph.D. Thesis, Université de Nancy I, France, 1983.
(7) Akin, E.W. *Water Sci. Technol.* **1984**, *17*, 689–700.
(8) "Human Viruses in the Aquatic Environment," EPA-570/9-78-006; EPA: Washington, D.C., 1978.
(9) O'Connors, J. T.; Hemphill, L.; Reach, C. D. "Removal of Virus from Public Water Supplies," Report PB 82-230-327; National

Technical Information Service: Springfield, Va., 1982.
(10) Stetler, R. E.; Ward, R. L.; Waltrip, S. C. *Appl. Environ. Microbiol.* **1984**, *47*, 319–24.
(11) Wellings, F. M.; Mountain, C. W.; Lewis, A. L. In *Proceedings of the Second National Conference on Individual Onsite Wastewater Systems;* National Sanitation Foundation: Ann Arbor, Mich. 1975; pp. 61–65.
(12) Hejkal, T. et al. *J. Am. Water Works Assoc.* **1982**, *74*, 318–21.
(13) Riggs, J. L.; Spath, D. P. "Viruses in Water and Reclaimed Wastewater," EPA-600/S1-83-018; EPA: Research Triangle Park, N.C., 1983.
(14) Deetz, T. R. et al. *Water Res.* **1984**, *18*, 567–72.
(15) Gerba, C. P. et al. "Virus Removal During Conventional Drinking Water Treatment," final project report on Grant CR809331010; EPA: Cincinnati, Ohio, 1984.
(16) Keswick, B. H. et al. *Appl. Environ. Microbiol.* **1984**, *47*, 1290–94.
(17) Sekla, L.; Stackin, W.; VanBuckenhout, L. *Can. J. Microbiol.* **1980**, *26*, 518–23.
(18) Payment, P. *Can. J. Microbiol.* **1981**, *27*, 417–20.
(19) Payment, P. et al. *Can. J. Microbiol.* **1984**, *30*, 105–12.
(20) Payment, P.; Trudel, M.; Plante, R. *Appl. Environ. Microbiol.* **1985**, *49*, 1418–28.
(21) Nupen, E. M. Presented at the Regional Conference on Water Supply and Pollution Control, South Africa, 1975.
(22) Nupen, E. M. In *Viruses in Water;* Berg, G. et al., Eds.; American Public Health Association: Washington, D.C., 1976; pp. 189–95.
(23) Grabow, W.O.K.; Nupen, E. M.; Bateman, B. W. Presented at the Symposium on Enteric Viruses in Water, Herzlia, Israel, December 1982.
(24) "Human Viruses in Water, Wastewater and Soils," Technical Report Series No. 639; World Health Organization: Geneva, Switzerland, 1979.
(25) Tyler, J. M. In *Viruses and Disinfection of Water and Wastewater;* Butler, M. et al., Eds.; University of Surrey Press: Surrey, U.K., 1982; pp. 42–63.
(26) Rabishko, E.V. *Gig. Sanit.* **1974**, *39*, 105–6.
(27) Kazantseva, V. A.; Drozdov, S. G. Presented at the Symposium Franco–Soviétique sur les problèmes de virologie médicale, Moscow, USSR, September 1980.
(28) Nestor, I. *Rev. Roum. Med. Virol.* **1979**, *30*, 155–56.
(29) Nestor, I. et al. *Rev. Roum. Med. Virol.* **1978**, *29*, 203–7.
(30) Walter, R.; Dobberkau, H. J.; Durkop, J. In *Viruses and Disinfection of Water and Wastewater;* Butler, M. et al., Eds; University of Surrey Press, Surrey, U.K., 1982; pp. 144–53.
(31) Kool, H. J.; Van Kranen, H. J. *Monogr. Virol.* **1984**, *15*, 134–45.
(32) Van Olphen, M. et al. *Appl. Environ. Microbiol.* **1984**, *47*, 927–32.
(33) Petrilli, F. L. et al. *Boll. Ist. Sieroter. Milan.* **1974**, *53*, 1–9.
(34) Pares, R.; Lucena, F.; Finance, C. *Cyma* **1980**, *13*, 86–90.
(35) Sexton, M.F.H.; Grohmann, G. S.; Murphy, A. M. "Infectious Gastroenteritis in Norfolk Island," Communicable Diseases Intelligence Bulletin No. 80/19; Commonwealth Department of Health: Australia, 1980.
(36) Murphy, A. M.; Grohmann G. S.; Sexton M.F.H. *J. Hyg.* **1983**, *91*, 139–46.
(37) Zhang, Chuyu et al. *J. Environ. Sci.* **1984**, *5*, 29–31.
(38) Rao, V. C.; Waghmare, S. V.; Lakke, S. B. *Appl. Environ. Microbiol.* **1981**, *42*, 421–26.
(39) Otatume, S.; Addy P.A.K. *Med. Biol. Tokyo* **1974**, *88*, 89–94.
(40) Bitton, G. et al. "Survey of Virus Isolation Data from Environmental Samples," report on Contract 68-03-3196; EPA: Cincinnati, Ohio, 1985.
(41) Farrah, S. R.; Bitton, G. In *Methods in Environmental Virology;* Gerba, C. P.; Goyal, S. M., Eds.; Marcel Dekker: New York, N.Y., 1982; pp. 117–49.
(42) Goyal, S. M; Gerba, C. P. In *Methods in Environmental Virology;* Gerba, C. P.; Goyal, S. M., Eds.; Marcel Dekker: New York, N.Y., 1982; pp. 59–116.
(43) Katzenelson, E.; Fattal, B.; Hostovesky, T. *Appl. Environ. Microbiol.* **1976**, *32*, 638–39.
(44) Sarrette, B. A.; Danglot, C. D.; Villagines, R. *Water Res.* **1977**, *11*, 355–58.
(45) Foliguet, J. M. et al. *Rev. Hyg. Med. Soc.* **1966**, *14*, 203–30.
(46) Walter, R. et al. *Z. Gesamte Hyg.* **1982**, *28*, 391–95.
(47) Ramia, S.; Sattar, S. A. *Appl. Environ. Microbiol.* **1980**, *39*, 49.
(48) Guttman-Bass, N.; Armon, R. *Appl. Environ. Microbiol.* **1983**, *45*, 850–55.
(49) Smith, E. M.; Gerba, C. P. *Appl. Environ. Microbiol.* **1982**, *43*, 1440–50.
(50) Sobsey, M. D. et al. *Water Sci. Technol.*, in press.
(51) *Indicators of Viruses in Water and Food;* Berg, G. Ed.; Ann Arbor Science: Ann Arbor, Mich., 1978.
(52) Bitton, G. *Introduction to Environmental Virology;* Wiley-Interscience: New York, N.Y., 1980.
(53) International Association of Water Pollution Research and Control Conference Study Group on Water Virology. *Water Res.* **1983**, *17*, 121–32.
(54) Berg, G.; Metcalf, T. G. In *Indicators of Viruses in Water and Food;* Berg, G., Ed.; Ann Arbor Science: Ann Arbor, Mich., 1978; pp. 267–96.
(55) Grabow, W.O.K.; Burger, J. S.; Nupen, E. M. *Prog. Water Technol.* **1980**, *12*, 803–17.
(56) "National Interim Primary Drinking Water Regulations," *Fed. Regist.* **1975**, *40*, 59566–574.
(57) American Water Works Association Committee. *J. Am. Water Works Assoc.* **1979**, *71*, 441–44.
(58) *Guidelines for Drinking-Water Quality. Recommendations;* World Health Organization: Geneva, Switzerland, Vol. 1, 1984.
(59) Shaffer, P.T.B.; Metcalf, T. G.; Sproul, O. J. *Appl. Environ. Microbiol.* **1980**, *40*, 1115–21.
(60) Berg, G.; Dean, R. B.; Dahling, R. B. *J. Am. Water Works Assoc.* **1968**, *60*, 193–97.
(61) Robeck, G. G.; Clarke, N. A.; Dostal, K. A. *J. Am. Water Works Assoc.* **1962**, *54*, 1275.
(62) Rudiger, St. et al. *Acta Hydrochim. Hydrobiol.* **1982**, *10*, 377–98.
(63) Walter, R.; Rudiger, St. Z. *Gesamte Hyg.* **1981**, *23*, 90–92.
(64) Payment, P.; Trudel, M.; Plante, R. *Eau du Quebec* **1983**, *16*, 251–54.
(65) Feachem, R.; Garelick, H.; Slade, J. *Trop. Dis. Bull.* **1981**, *78*, 185–230.
(66) Gamble, D. R. *Lancet* **1979**, *8113*, 425–28.
(67) Foliguet, J. M. *Eau* **1983**, *1–2*, 3–9.
(68) Sobsey, M. D. *J. Am. Water Works Assoc.* **1975**, *67*, 414–18.
(69) Goldfield, M. Presented at the International Conference on Viruses in Water, Mexico City, Mexico, 1974.
(70) Minor, T. E. et al. *J. Clin. Microbiol.* **1981**, *13*, 388–89.
(71) Ward, R. L.; Akin, E. W. *Crit. Rev. Environ. Control* **1984**, *14*, 297–310.
(72) Gerba, C. P.; Singh, S. N.; Rose, J. B. *Crit. Rev. Environ. Control* **1985**, *15*, 213–36.
(73) Batik, O. et al. *Am. J. Public Health* **1980**, *70*, 167–68.
(74) Craun, G. F.; McCabe, L. J. *J. Am. Water Works Assoc.* **1973**, *65*, 74–84.
(75) Neefe, J. R. et al. *J. Am. Med. Assoc.* **1945**, *128*, 1076–79.
(76) Grabow, W.O.K. et al. *Appl. Environ. Microbiol.* **1983**, *46*, 619–24.
(77) Peterson, D. A. et al. *Appl. Environ. Microbiol.* **1983**, *45*, 223–27.
(78) Kaplan, J. E. et al. *Am. J. Public Health* **1982**, *72*, 1329–32.
(79) Kaplan, J. E. et al. *J. Infect. Dis.* **1982**, *146*, 190.
(80) Taylor, J. W.; Gary, G. W.; Greenberg, H. B. *Am. J. Epidemiol.* **1981**, *114*, 584.
(81) Birch, C. J. et al. *J. Med. Virol.* **1983**, *11*, 241–44.
(82) Sato, K. et al. *Arch. Virol.* **1981**, *69*, 155.
(83) Clark, R. M.; Goodrich, J. A.; Ireland, J. C. *J. Environ. Syst.* **1984**, *14*, 1–30.
(84) Baker, E. L. et al. *Am. J. Public Health* **1979**, *69*, 501–2.

***Gabriel Bitton** (l.) is a professor in the Department of Environmental Engineering Sciences at the University of Florida, Gainesville. He has conducted research on surface microbiology and on the interactions of viruses with surfaces. Bitton has published extensively in the field of environmental virology and is the author of a book,* Introduction to Environmental Virology *(Wiley, 1980).*

***Samuel R. Farrah** (r.) is an associate professor in the Department of Microbiology and Cell Sciences at the University of Florida. He has conducted research on virus adsorption to solids and on the fate of microorganisms in sludge during aerobic digestion.*

***Clay L. Montague** (l.) is a systems ecologist in the Department of Environmental Engineering Sciences at the University of Florida. His interests include computer simulation of complex resource management issues, especially within estuarine ecosystems. He has a Ph.D. in zoology from the University of Georgia, an M.S. from Georgia Tech, and a B.S. from the University of North Carolina.*

***Elmer W. Akin** (r.) was a commissioned officer in the U.S. Public Health Service and was detailed to the EPA when it was created in 1970. He now serves as the acting director of the EPA Toxicology and Microbiology Division Health Effects Research Laboratory. Akin has 50 publications and scientific presentations in the field of environmental virology to his credit.*

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